

AMENDMENTS TO THE CLAIMS

**COMPLETE LISTING OF ALL CLAIMS, WITH MARKINGS AND STATUS IDENTIFIERS**  
(Currently amended claims showing deletions by ~~striketrough~~ and additions by underlining)

This listing of claims will replace all prior versions and listings of the claims in the application.

Listing of Claims:

Claims 1-60. (canceled)

61. (new) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; and/or

(b) a lethal gene, wherein expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

62. (new) The cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment.

63. (new) The cell of claim 61 wherein the permissive environment comprises an environment containing a nutrient required to maintain expression of the essential gene, prevent expression of the lethal gene, or both, and the non-permissive environment comprises an environment lacking the nutrient.

64. (new) The cell of claim 61 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

65. (new) The cell of claim 61 wherein the lethal gene is carried on an extrachromosomal vector.

66. (new) The cell of claim 61 wherein the lethal gene is carried on a chromosome of the cell.
67. (new) The cell of claim 61 wherein the essential gene comprises the *asd* gene operatively linked to *araC*-P<sub>BAD</sub>.
68. (new) The cell of claim 65 wherein the vector has two lethal genes.
69. (new) The cell of claim 68 wherein the vector comprises pMEG-104.
70. (new) The cell of claim 61 wherein the cell is a gram-negative bacterium.
71. (new) The cell of claim 70 wherein the gram-negative bacterium is an enteric bacterium.
72. (new) The cell of claim 71 wherein the genus of the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.
73. (new) The cell of claim 61 wherein the essential gene is carried on an extrachromosomal vector.
74. (new) The cell of claim 73 wherein expression of the essential gene is regulated by an expression product of a regulatory gene on a chromosome of the cell.
75. (new) The cell of claim 73 wherein expression of the essential gene is regulated by an expression product of a regulatory gene on an extrachromosomal vector.
76. (new) The cell of claim 61 wherein the essential gene is carried on a chromosome of the cell.
77. (new) The cell of claim 61 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

78. (new) The cell of claim 77 wherein expression of the replication gene is regulated by an expression product of a regulatory gene.

79. (new) The cell of claim 61 wherein replication of the extrachromosomal vector is regulated by an expression product of a regulatory gene,

wherein the absence of a functional expression product of the regulatory gene derepresses expression of the replication gene and wherein the expression product is not expressed or is inactive only in the non-permissive environment.

80. (new) The cell of claim 61 further comprising an expression gene wherein the expression gene encodes a desired expression product.

81. (new) The cell of claim 61 further comprising a transfer vector.

82. (new) The cell of claim 61 wherein the lethal gene is a gene required for excision of a prophage, wherein the prophage is in the chromosome of the cell, and wherein excision of the prophage causes lysis of the cell.

83. (new) The cell of claim 61 wherein the essential gene, the lethal gene, or both have engineered expression.

84. (new) A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

(a) an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; and/or

(b) a lethal gene, wherein expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

85. (new) The method of claim 84 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

86. (new) The method of claim 84 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

87. (new) The method of claim 84 wherein the cell is temporarily viable in the non-permissive environment.

88. (new) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein said essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

89. (new) The cell of claim 88 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

90. (new) The cell of claim 88 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal,  
wherein the cell is a member of the *Enterobacteriaceae*.

91. (new) The cell of claim 88 wherein the essential gene is carried on an extrachromosomal vector.

92. (new) The cell of claim 88 wherein the cell is a gram-negative bacterium.

93. (new) The cell of claim 92 wherein the gram-negative bacterium is an enteric bacterium.
94. (new) The cell of claim 93 wherein the genus of the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.
95. (new) The cell of claim 88 wherein expression of the essential gene is regulated by the expression product of a regulatory gene.
96. (new) The cell of claim 95 wherein the expression product of the regulatory gene inhibits expression of the essential gene and is expressed or active only in the non-permissive environment.
97. (new) The cell of claim 91 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.
98. (new) The cell of claim 88 further comprising an expression gene wherein the expression gene encodes a desired expression product.
99. (new) A method of inducing an immune response in a warm-blooded animal comprising  
administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising  
an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and  
wherein the essential gene corresponds to an inactivated native gene of the cell, wherein the cell is a member of the *Enterobacteriaceae*.

100. (new) The method of claim 99 wherein the system further comprises an expression gene wherein the expression gene encodes an antigen.

101. (new) The method of claim 100 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

102. (new) The method of claim 100 wherein the essential gene is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, wherein the cell is a member of the *Enterobacteriaceae*.

103. (new) The cell of claim 95 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

104. (new) The cell of claim 88 wherein the essential gene is a gene essential for metabolism or growth of the cell.

105. (new) The cell of claim 88 wherein the essential is a gene essential for cell wall or cell membrane integrity.

106. (new) The cell of claim 88 wherein the essential gene is a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP.

107. (new) The method of claim 88 wherein the essential gene is an *asd* gene, a *dap* gene, a *dal* gene, a *ddl* gene, a *fab* gene, or a *pls* gene.